

Fouchet Reagent for Bile Stain, Hall's Method – Technical Memo

<u>SOLUTIONS:</u>	250 ml	500 ml
Fouchet Reagent	Part 1095A	Part 1095B

Additionally Needed:

Bile Control Slides	Part 4060
Van Gieson Stain	Part 1404
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842

For storage requirements and expiration date refer to individual product labels.

APPLICATION:

Newcomer Supply Bile Stain, Hall's Method is for the demonstration of bile (bilirubin) substances in tissue sections and to distinguish bile pigments from other tissue pigments. The acidity of Fouchet Reagent works to oxidize bilirubin to biliverdin, resulting in a green color development with the Van Gieson Stain serving as a complimentary counterstain.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)

Technique: Paraffin sections cut at 4 microns

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure.

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Filter Fouchet Reagent with Grade 1 filter paper prior to use.

STAINING PROCEDURE:

3. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - a. See Procedure Notes #1 and #2.
4. Place slides in freshly filtered Fouchet Reagent for 5 minutes.
5. Wash in three changes of tap water; rinse in distilled water.
6. Stain sections in Van Gieson Stain (1404) for 5 minutes.
7. Rinse quickly in 95% ethyl alcohol.
8. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Bile/bilirubin	Emerald green to olive drab
Connective tissue	Pink to red
Background	Yellow

PROCEDURE NOTES:

1. Drain slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during procedure.
3. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 268-269.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 219.
3. Modifications developed by Newcomer Supply Laboratory.